

# Olfactory Cues in Host Finding by *Melittobia digitata* (Hymenoptera: Eulophidae)

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**ABSTRACT** Chemical signals used by parasitoids to find hosts often originate from the host, its habitat, or both, providing critical cues for locating hosts that are often cryptic or highly dispersed. *Melittobia* Westwood are gregarious ectoparasitoids, which primarily attack *Trypoxylon politum* Say prepupae. How *Melittobia* locates its host is unknown, but it may involve host-related chemical signals. Therefore, this study focused on whether host location by *Melittobia digitata* Dahms is mediated by olfactory stimuli. In a small arena, which contained a choice of potential hosts [*T. politum* prepupa, *Megachile rotundata* (F.) prepupa, or *Sarcophaga bullata* (Parker) puparium], empty host pupal cases, or nest mud—all of which were visually and physically isolated from the parasitoid—*Mel. digitata* successfully located host patches and spent significantly more time on those than on control (blank and dummy) patches. Results suggest that *Mel. digitata* females may be arrested by host-related chemicals.

**KEY WORDS** parasitism, chemoreception, host location, flesh fly, leafcutting bee

PARASITOIDS OF THE GENUS *Melittobia* (Mel.) Westwood (Hymenoptera: Eulophidae) are small, cosmopolitan, gregarious external parasitoids. In their natural host range, they primarily parasitize solitary wasps and bees and theirinquilines (Freeman and Ittyeipe 1982, Matthews et al. 1985). *Melittobia* show remarkable plasticity of behavior, and theoretically, unmated females can survive and eventually produce progeny of both sexes, even in the absence of preferred hosts (Dahms 1984).

In the laboratory, *Melittobia* accepts a variety of hosts, including species of Diptera and Coleoptera (Thompson and Parker 1927). In nature, this host range probably serves *Melittobia* well, enabling it to reproduce on many of the other parasites that often infest a solitary wasp or bee nest (Matthews et al. 1996).

After finding and entering the nest of a developing host, a mated *Melittobia* female will lay hundreds of eggs on the host surface. If the food supply is low, she may seek another host to complete oviposition, usually moving to a neighboring host cell by chewing through the cell wall (Dahms 1984). How these species locate, recognize, and assess their host is unknown and little studied (Trexler 1985, Ranger 1996). Therefore, we investigated whether female parasitoids *Mel. digitata* use host-produced olfactory cues to locate a host, either its natural host *Trypoxylon politum* Say (Hymen-

optera: Sphecidae) prepupae or laboratory alternative hosts, puparia of the flesh fly, *Sarcophaga bullata* (Parker) (Diptera: Sarcophagidae), and prepupae of the alfalfa leafcutting bee, *Megachile* (Meg.) *rotundata* (F.) (Hymenoptera: Megachilidae), which are suitable alternative hosts (Matthews et al. 1996, González and Matthews 2002, Silva-Torres and Matthews 2003).

Our hypothesis was if *Mel. digitata* females find their hosts by means of olfactory cues, then inside a choice arena it is more likely that they will move toward a host odor source (nude hosts, host enclosed in cocoons, host puparia, host cocoons, nest mud, and host/cocon extracts) than to a dummy or randomly.

## Materials and Methods

This study was conducted at Coastal Plain Experiment Station in Tifton, GA, Laboratory of Biological Control. Tests were carried out at normal laboratory temperature ( $25.2 \pm 0.46^\circ\text{C}$ ) and humidity ( $41 \pm 1.41\%$ ).

**Subjects.** This study used *Mel. digitata* females obtained from cultures maintained in the Laboratory of Insect Behavior of the University of Georgia, Athens, GA. They were cultured on three hosts: *T. politum* prepupa, *S. bullata* puparium, and *Meg. rotundata* prepupa in different colonies according to specific host. *Trypoxylon* prepupae were collected from nests around Athens and Tifton, GA. Meanwhile, *S. bullata* puparia were obtained from Carolina Biological Supply Company, and *Meg. rotundata* cocoons from Pioneer Hi-Bred International, Inc., respectively. Parasitoid colonies were kept in rearing chambers at  $25^\circ\text{C}$  for

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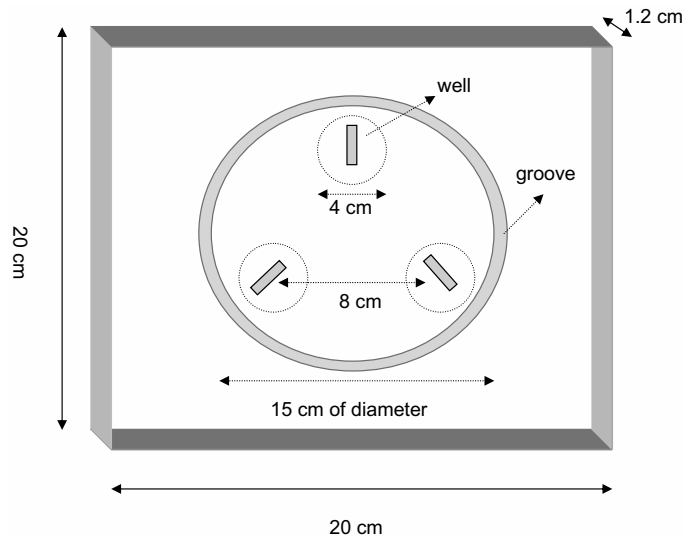


Fig. 1. Base of choice arena used in this study. Top was a petri dish 15 cm in diameter (not shown). One well of the base was filled with a host treatment; the second with a piece of glass rod (dummy), and the third was empty (the blank control). The wells and the entire arena were covered with a single piece of filter paper, and one parasitoid female was introduced in the center of the arena for each trial. A female was considered to have responded upon entering the respective small patch (dotted line) surrounding each well. When extracts were used as odor source they were applied on the filter paper within the same patch around the well.

24-h darkness with no food or water provided because these wasps obtain their food supply by feeding on the host (Dahms 1984). Females *Mel. digitata* usually engage in courtship behavior with their brothers (Matthews et al. 1996). Therefore, parasitoids were allowed to stay with the natal host until 1 h before the trials to ensure mating, and then they were transferred to clean glass vials.

**Bioassay Setup and Protocols.** We used a three-way choice arena to address our questions (Fig. 1). This arena apparatus consisted of two parts: a Plexiglas base (20 by 20 by 1.2 cm) and a glass top (bottom of a petri dish of 15-cm diameter, Pyrex). A groove of the same circumference was routed into the base to receive the glass top. The Plexiglas base contained three rectangular depressions or wells (1 by 2 by 1 cm), arrayed in an equilateral triangle with sides 8 cm in length.

By random assignment, one well of the choice arena received one of several experimental materials—either a live host (*T. politum* or leafcutting bee prepupa or fly puparium), cocoon material from one of these hosts (except for flesh fly), cocoon extract, prepupa extract, or mud fragments from a *T. politum* nest. A second well was given a dummy (piece of glass rod of same approximate size as a host). The third well of the arena remained empty. The blank was used as a control for the dummy because past studies involving *Mel. digitata* host acceptance have shown that pupa-shaped glass objects were significantly more accepted by the parasitoid females than rectangular pieces of glass and extract-treated glass (Cooperband and Vinson 2000). A circular filter paper 20 cm in diameter (Whatman, Maidstone, England), placed over the arena base covered the wells to remove visual and physical cues. It

was held taut by pressing it into the routed groove in the apparatus with the rim of the petri dish. This also effectively sealed the interior of the arena preventing wasps from escaping or physically contacting a host. A single mated, inexperienced macropterous *Mel. digitata* female (<5 d old and emerged from specific host being offered) was first released onto the center of the filter paper with the help of a paint brush, and then the lid was pressed tightly into the base groove closing the arena. Finally, because female *Melittobia* show negative geotactic behavior (Guinan and Matthews 2000), the arena was inverted and suspended above a viewing mirror from which each tested female parasitoid's movements could be tracked. To minimize possible light influences on the parasitoid's behavior, all trials were conducted in the dark. Wasps were observed using a flashlight covered by red cellophane (presumably red light is not perceived by this insect).

For the first minute after introduction into the arena, the female was allowed to acclimate to the new dark surroundings. Additionally, preliminary tests in this apparatus showed that females did not immediately begin to orient toward any of the offered treatments. Therefore, beginning at the second minute, the female's first treatment choice and the time (in seconds) spent in each treatment patch of the arena were recorded over the next 20 min (Fig. 1). Twenty trials, each using a new female of standard age from the same source culture, were run. Upon introduction of each female into the apparatus, which had to be opened by removing the lid, the system was aerated and the effect of prior air saturation inside the arena was eliminated. The arena was rotated 130° after each trial to avoid possible treatment position bias. The host treatment,

Table 1. Mean time (s) spent by single, mated, inexperienced *Mel. digitata* females during a 20-min trial responding to various combinations of *T. politum* hosts compared with controls

Treatment	No. of females responding out of 20 tested	Mean time $\pm$ SE	Compared with dummy and blank controls ( $F_{df}$ ) <sup>a</sup>
Mud + cocoon + prepupa	15.0	262.8 $\pm$ 35.99	$F_{2, 44} = 33.43^*$
Controls avg <sup>b</sup>	16.0	46.8 $\pm$ 7.65	
Cocoon	19.0	377.6 $\pm$ 28.17	$F_{2, 50} = 115.85^*$
Controls avg	17.0	46.7 $\pm$ 7.33	
Nude prepupa	17.0	144.0 $\pm$ 33.33	$F_{2, 44} = 2.73$ ; $P = 0.0762$
Controls avg	15.0	59.9 $\pm$ 9.99	
Cocoon extract	18.0	138.9 $\pm$ 19.36	$F_{2, 51} = 14.68^*$
Controls avg	17.5	48.9 $\pm$ 6.75	
Nude prepupa extract	14.0	73.0 $\pm$ 17.80	$F_{2, 42} = 1.13$ ; $P = 0.3322$
Controls avg	15.5	60.51 $\pm$ 9.42	
Mud	18.0	145.6 $\pm$ 32.9	$F_{2, 51} = 8.14$ ; $P = 0.0009$
Controls avg	17.0	42.7 $\pm$ 4.18	

<sup>a</sup> For all treatments followed by an asterisk,  $P < 0.0001$ .

<sup>b</sup> The amount of time females spent in the control patches, dummy and blank, was not significantly different from each other, regardless of the host treatment offered. Therefore, only the average of the two control treatments is shown.

dummy, and blank were rotated among the wells after every third trial when the filter paper was changed and the arena washed with water and 70% alcohol. Females were considered as "responding" to a treatment when they entered, at least once, the arena patch containing the respective treatment under the filter paper.

To obtain host extracts, a single host cocoon or prepupa was washed with 5 ml of hexane (Sigma, St. Louis, MO) for 2 min at room temperature. Extracts were stored in 7-ml scintillation vials (Solvent Saver, Kimble Kontes, Vineland, NJ) and put in the freezer until further experimental procedure, when 80  $\mu$ l of the extract was pipetted onto a 4-cm-diameter disk of filter paper (Fig. 1) and allowed to evaporate for 10 min before running a trial. A pure hexane-treated filter paper patch served as control for the solvent.

**Data Analysis.** The time spent by responding parasitoid females around the respective treatment patches in the arena was tested for normality by using Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von, and Anderson tests (SAS Institute 2000). Most of the parameters showed significant deviation from a normal distribution according to results of three or all normality tests applied. Hence, they were transformed into square root ( $x + 0.5$ ) to meet analysis of variance (ANOVA) assumptions. Then, data were analyzed using one-way ANOVA (PROC ANOVA, SAS Institute 2000) and Waller-Duncan's test of mean comparisons (SAS Institute 2000). Data for the first choice made by females, and the number of responding females to a treatment or controls was analyzed using a  $\chi^2$  test (PROC FREQ, SAS Institute 2000).

## Results

Individual females of *Mel. digitata* were able to successfully locate host patches hidden under the filter paper and spent significantly more time on these patches than on nonhost, control patches in the three-way choice arena. Initially, most *Mel. digitata* females spent considerable time walking about inside the apparatus, but once they crossed the boundaries of the wells that contained the hosts, they were considered

to have responded, and they spent significantly more time investigating such areas. Overall, female parasitoids spent more time in areas containing either a *Meg. rotundata* prepupa inside its cocoon or a *T. politum* cocoon than in any other treatment offered (Tables 1 and 2). Generally, the average number of females responding to the controls compared with the number responding to the treatments was not different, except for the three treatments involving *Meg. rotundata* in which significantly greater numbers of females responded to the treatment well than to the control well average (Table 2:  $\chi^2 = 9.2308$ ,  $P = 0.0024$ ;  $\chi^2 = 5.7143$ ,  $P = 0.01669$ ; and  $\chi^2 = 4.80$ ,  $P = 0.0285$ ; respectively).

Empty cocoons from *T. politum* and *Meg. rotundata* were significantly more attractive to *Mel. digitata* females than the controls, indicating that chemicals present in these structures may play an important role in *Mel. digitata* initial close-range attraction (Tables 1 and 2).

Females spent significantly more time on arena patches that contained *T. politum* and *Meg. rotundata* cocoon extracts, respectively, than on control patches; however, time spent on *T. politum* cocoon extract was lower than the time spent on the real cocoon patch (Table 1).

Similarly, *Mel. digitata* spent significantly more time on patches that contained mud from the *T. politum* nest than on control patches (Table 1). In addition, the time spent by the females on nest mud patches and time they spent on patches containing nude *T. politum* prepupa was similar, suggesting that, even though they are different host treatments, they might be equally "attractive" to the wasps.

Results of *Mel. digitata* female first choice in the arena showed that even though they can respond to host-related chemicals, the initial distribution movement of responding females is random (Fig. 2). Overall, the number of times they chose a host as first choice was not significantly different from the number of times they chose the controls, except for the *Meg. rotundata* cocoon, which was more frequently chosen first by the females (Fig. 2).

Table 2. Mean time (s) spent by single, mated, inexperienced *Mel. digitata* female during a 20-min trial responding to *Meg. rotundata* (various combinations), *S. bullata*, or hexane extracts compared with controls

Treatment	No. of females responding out of 20 tested	Mean time ± SE	Compared with dummy and blank controls ( $F_{df}$ ) <sup>a</sup>
<i>Meg. rotundata</i>			
Cocoon + prepupa	18.0	465.6 ± 70.69	$F_{2, 33} = 22.72^*$
Controls avg <sup>b</sup>	9.0	68.6 ± 16.04	
<i>Meg. rotundata</i>			
Cocoon	20.0	247.7 ± 37.90	$F_{2, 47} = 26.38^*$
Controls avg	15.0	57.0 ± 6.27	
<i>Meg. rotundata</i>			
Cocoon extract	18.0	238.9 ± 50.09	$F_{2, 39} = 11.3^*$
Controls avg	12.0	63.6 ± 9.91	
<i>S. bullata</i>			
Puparia	15.0	234.5 ± 41.85	$F_{2, 40} = 14.99^*$
Controls avg	14.0	59.1 ± 12.19	
Hexane	19.0	84.1 ± 11.23	$F_{2, 51} = 1.92; P = 0.1573$
Controls avg	17.5	57.8 ± 6.73	

<sup>a</sup> For all treatments followed by an asterisk.  $P < 0.0001$ .  
<sup>b</sup> The amount of time females spent in the control patches, dummy and blank, was not significant different from each other, regardless of the host treatment offered. Therefore, only the average of these control treatments is shown.

Discussion

The results provide strong support for chemical cues mediating close-range host seeking behavior by *Mel. digitata* because parasitoid females successfully recognized and located host patches (*T. politum*, *Meg. rotundata*, and *S. bullata*) hidden under the filter paper and spent significantly more time on these patches than on nonhost (blank and dummy), control patches. These results and those reported by Ranger (1996) agree with the hypothesis that *Mel. digitata* females use olfactory stimuli to locate their hosts. Trexler (1985) observed nonrandom selection of host-containing nests by *Melittobia* females, evidence that they likely use some sort of odor cues to locate hosts at close range.

Although leafcutting bees, *Meg. rotundata*, have yet to be reported as a natural host for *Mel. digitata* in the field, other species of *Melittobia* such as *Mel. australica* Girault, *Mel. acasta* (Walker), and *Mel. hawaiiensis* Perkins have been found parasitizing this bee species (Peck 1969, MacFarlane and Donovan 1989, Woodward 1994). Additionally, *Meg. rotundata* is successfully used as an alternative host for *Mel. digitata* in the laboratory (González and Matthews 2002). That *Mel. digitata* spent more time on *Meg. rotundata* patches than any other host or nest material offered in the arena trials suggests that this parasitoid could become an additional problem for alfalfa growers if it becomes a field parasitoid of *Meg. rotundata*, currently the most widely used commercially managed pollinator, after

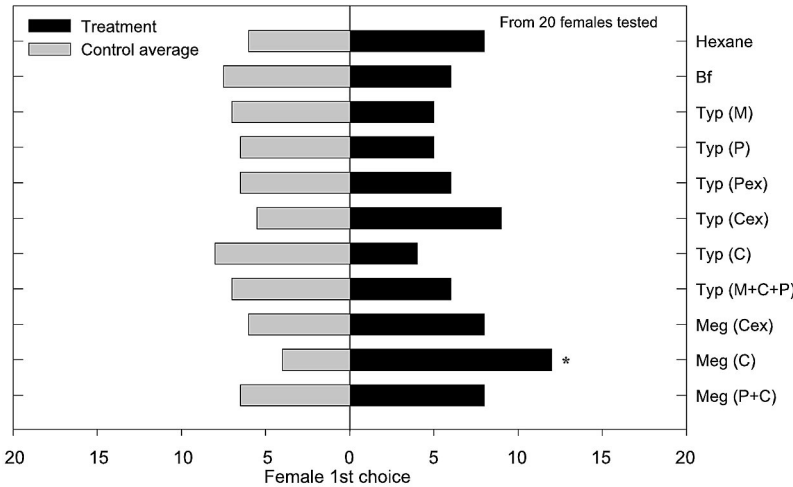


Fig. 2. Frequencies of first choice made by responding single mated inexperienced *Mel. digitata* females tested in an arena containing a possible host and controls (average of dummy and blank) during a 20-min trial ( $n = 20$ ). Bar followed by an asterisk is significantly different from the control ( $\chi^2 = 0.2857, P = 0.0455$ ). Mud + cocoon + *Trypoxylon* prepupae [Typ (M+C+P)]; naked *Trypoxylon* prepupa [Typ (P)]; *Trypoxylon* empty cocoon [Typ (C)]; *Trypoxylon* cocoon extract [Typ (Cex)]; *Trypoxylon* naked prepupae extract [Typ (Pex)]; mud [Typ (M)]; *Sarcophaga bullata* puparia [Bf]; *Megachile* cocoon extract [Meg (Cex)]; *Megachile* prepupa + cocoon [Meg (P+C)]; and *Megachile* empty cocoon [Meg (C)].



the honey bee, *Apis mellifera* L. (Kemp and Bosch 2000). Donovan et al. (1982) found that *Mel. hawaiiensis* parasitized *Meg. rotundata* prepupae in New Zealand within 8 wk of release of leafcutting bees in the field and that the parasitism rate increased from 0.02 to 11.3% in just 3 yr. Similarly, Woodward (1994) reported that *Mel. australica* parasitized 19% of *Meg. rotundata* population in South Australia in 1988–1989.

Positive responses of *Mel. digitata* toward flesh fly puparia in the arena could be attributed to flesh flies being extensively used in laboratory cultures of *Melittobia* as an alternative host yielding reasonable numbers of progeny per female (Matthews et al. 1996, Silva-Torres and Matthews 2003). Chemically, flesh fly puparia are likely to share similarities with certain dipteran inquilines, such as satellite flies (various Sarcophagidae: Miltogramminae) and bee flies (Bombyliidae: *Anthrax* sp.), which often occur in *T. politum* nests and also can be successfully parasitized by *Melittobia* (Dahms 1984, Matthews et al. 1996).

Female *Mel. digitata* also responded positively to nest mud compared with the controls; however, this response was relatively low and similar to the response of females toward naked prepupae. Thus, these results suggest that chemicals from both mud and nude *T. politum* prepupae have a lesser role in *Mel. digitata* host finding and recognition in comparison with host cocoons. However, when offered as found in the natural nest, (i.e., nest mud + cocoon + prepupa), the combined chemical bouquet seems to enhance host location and recognition.

Host cocoons and their extracts were significantly more attractive to *Mel. digitata* (Tables 1 and 2) than the controls; however, *Mel. digitata* response toward cocoon extracts was considerably lower than that elicited by the cocoon itself (for *T. politum*). This could be due to the extraction method (possibly some essential components were not extracted with the hexane) or to degradation that occurred during freezer storage before use. Ranger (1996) reported similar results when experienced *Mel. digitata* females were offered *T. politum* cocoons. According to Ranger, cocoon surface hydrocarbons play a major role in arresting searching behavior in *Melittobia* females. Because viable *T. politum* prepupae in their cocoons are usually collected and stored in the refrigerator for extended periods before use, volatiles may be lost. In our study, *T. politum* nests were harvested and tested or extracted during the same nesting season. Presumably any cocoon surface chemicals used in host location by *Mel. digitata* would still have been present and readily detectable. Gas chromatography-mass spectrometry analysis (Ranger 1996) revealed that *T. politum* cocoon hydrocarbons are simple, common n-alkanes having 23–29 carbon atoms. Similar hydrocarbons can be found in cocoons of other sphecids, e.g., *Sceliphron* spp. (Ranger 1996), also common hosts for *Melittobia*.

Research involving other parasitoid species also has indicated that cocoons may offer potential cues to a searching parasitoid female. For example, Weseloh (1988) reported that empty host cocoons of *Cotesia*

*melanoscela* (Ratzeburg) were attractive to its hyperparasitoid *Eurytoma appendigaster* (Swederus).

Interestingly, Trexler (1985) showed that the rate of *T. politum* host location by *Melittobia* in a Plexiglas arena decreased with increasing host densities. One might have predicted that higher densities of hosts would release more host-related volatiles that in turn would serve to attract more parasitoids. However, Price (1975) proposed that, because some parasitoid females can recognize odors of conspecifics, under restricted conditions (e.g., in a parasitized cocoon or an experimental arena), groups of female parasitoids would be more engaged in avoidance of each other and escape than in finding a host. This would result in a reduced number of parasitized hosts as parasitoid density increased.

Our results and those of others (Freeman and Parnell 1973, Taffe and Ittyeipe 1976, González and Terán 2001) have shown that *Mel. digitata* females initially seem to disperse more or less randomly (Fig. 2). Initial movement in the assay chamber was apparently undirected, but once a host odor was encountered females tended to spend more time in that region. Therefore, overall results suggest that wasp arrestment rather than attraction may be operating here. Curiously, the number of females responding to the various combinations of *Meg. rotundata* (Table 2, first three experiments) was significantly higher than those responding to the controls. This result suggests that *Meg. rotundata* somehow is differentially attracting females and is reinforced by the finding that the only significant difference in first choice was to *Meg. rotundata* cocoons (Fig. 2). Why *Meg. rotundata* should elicit so much stronger responses than the normal host, *T. politum*, is unknown.

In another study using *Mel. digitata*, when wasps were offered the same host treatments against controls in an olfactometer, they initially showed undirected movement (C.S.A.S.-T., unpublished data). Perhaps, after emergence and mating, long-winged females disperse to search for suitable hosts only in nearby areas such as neighboring cells and nests. If females perceive chemicals emanating from hosts and their cocoons, they respond to those odors perhaps by increased random turning (klinokinesis) and readily find the host. Failing to be arrested by host odors, females may change to a long-range dispersal behavior. Prior studies have suggested that, due to their small size, there likely could be passive dispersal induced by wind (Freeman and Parnell 1973, Taffe and Ittyeipe 1976, González and Terán 2001). However, further studies are needed to understand dispersal.

In summary, olfactory signals emanating from potential host cocoons seem to be important as short-range cues used by *Mel. digitata* females to locate acceptable hosts. The identity and chemical characterization of these cues remain to be investigated. Because of the taxonomic diversity of their hosts it seems likely that the host recognition odors will be mixtures of simple hydrocarbons common to a broad range of holometabolous species.

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